HELDA
HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

UNIVERSITY OF HELSINKI

https://helda.helsinki.fi

Small-scale spatial and temporal variation of life-history traits of common frogs (Rana temporaria) in sub-Arctic Finland

Cogalniceanu, Dan

2017-08

Cogalniceanu , D , Bancila , R I , Plaiasu , R , Rosioru , D & Merila , J 2017 , ' Small-scale spatial and temporal variation of life-history traits of common frogs (Rana temporaria) in sub-Arctic Finland ' , Polar Biology , vol. 40 , no. 8 , pp. 1581-1592 . https://doi.org/10.1007/s00300-017-2081-8

http://hdl.handle.net/10138/307938
https://doi.org/10.1007/s00300-017-2081-8

unspecified
acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Small-scale spatial and temporal variation of life history traits of common frogs (*Rana temporaria*) in sub-Arctic Finland

Cogălniceanu D[1], Băncilă RI[1,2], Plăiaşu R[1,2], Roşioru D[3], Merilä J[4]

[1] University Ovidius Constanţa, Faculty of Natural Sciences and Agricultural Sciences, Al. Universităţii 1, corp B, 900740 Constanţa, Romania

[2] "Emil Racoviţă" Institute of Speleology of Romanian Academy, 13 Septembrie Road, No. 13, 050711, Bucharest, Romania

[3] National Institute for Marine Research and Development "Grigore Antipa", Blvd. Mamaia 300, Constanţa, Romania

[4] University of Helsinki, Ecological Genetics Research Unit, Department of Biosciences, FI-00014 Helsinki, Finland

Corresponding author: Raluca I. Băncilă

"Emil Racoviţă" Institute of Speleology of Romanian Academy

13 Septembrie Road, No. 13, 050711, Bucharest, Romania

Tel: + 40 021 3188132; Fax: + 40 021 3188132

e-mail: raluca.bancila@ieu.uzh.ch

Abstract

Small-scale spatial and temporal variation in abiotic and biotic factors can cause variation in several major life history traits in vertebrate ectotherms such as amphibians. However, relatively little is known about small-scale variation in life history traits of sub-Artic amphibians. We studied the spatio-temporal variation of adult life history traits linked to age and body size in the common frog (*Rana temporaria*) from low (i.e., valley at 480 m a.s.l.) and high (i.e., hill at 530-650 m a.s.l.) altitude sites in the sub-Arctic Kilpisjärvi area (Finland). Data on life history traits of frogs from hill sites collected during a three-year field study were compared with previously published data from the valley sites. The results showed spatio-temporal variation in life-history traits, frogs responding to spatio-temporal variation in the environmental conditions with variation in age, life span, survival rates, body size and mass. Frogs from hill sites had shorter life span, both in terms of mean age (5.6 versus 10.5 years) and longevity (9-10 versus 18 years), smaller snout-vent length (63 versus 77 mm) and body mass (24 versus 45 g) than frogs from valley sites. The differences were more pronounced in females than in males indicating some sex-specific responses to environmental differences among sites. The results show that small differences in elevation (or elevation related abiotic and biotic factors) can translate to large differences in mean values of important life history traits in common frogs living at the edge of their distribution range.

Key words

spatio-temporal variation, life history traits, skeletochronology, age structure

Introduction

Geographic differentiation of life history traits and morphological features are of common occurrence in both plants and animals (Mayr 1963; Endler 1977; Linhart and Grant 1996; Roff 1992). Local populations at the limit of the species' range are thought to be under severe selection pressures to adapt and stay adapted to harsh environmental conditions they face (e.g. Aitken et al. 2008; Hill et al. 2011). This can be accomplished either through phenotypic plasticity, genetic adaptation, or both (Gienapp et al. 2008; Moritz and Agudo 2013). Exploring these mechanisms that allow a species to persist in challenging environments is crucial for understanding how species deal with ecological selection pressures (Merilä and Hendry 2014; Ludwig et al. 2015).

Widely distributed species can be of particular interest in this context because: i) they experience variation in climate, habitat and resource availability and quality over a wide geographic range; ii) their populations inhabiting distinct locations experience different ecological and climatic pressures and iii) these different selection pressures generate and maintain phenotypic differentiation in their life history traits (Bulgarella et al. 2015; Miaud and Merilä 2001). Ectothermic animals like amphibians are very sensitive to temperature as it influences directly their metabolism and sets limits to their distribution (Ludwig et al. 2015). Some temperate zone amphibians, such as the common frog (*Rana temporaria*), are widely distributed and provide excellent models for studying age- and size-related life-history traits (and potential trade-offs among them) across their wide latitudinal and altitudinal distribution range (Morrison and Hero 2003; Sinsch et al. 2015).

The common frog is considered to be a generalist anuran species in its habitat use (Van Buskirk and Arioli 2005) although other studies indicated habitat selectivity (Plăiaşu et al. 2010; Cogălniceanu et al. 2012). It is a widespread species occurring throughout most of Europe from northern Spain through North Cape to the Urals, and can be found at elevations ranging from sea level to 2,700 m a.s.l. To the south its

distribution becomes patchy and increasingly restricted to mountainous regions (Kuzmin et al. 2009). Due to its wide distribution range and high abundance, *R. temporaria* has been used as a model for testing a variety of hypotheses of ecological and evolutionary interest (e.g. Miaud and Merilä 2001; Sinsch et al. 2015). Both larval and adult life history traits display extensive geographic variation (Miaud et al. 1999; Miaud and Merilä 2001; Sinsch et al. 2015).

The common frog populations inhabiting Fennoscandia have been subject to several studies focused on geographic variation along a latitudinal gradient. They have been used in testing Bergmann's rule in adult body size (Laugen et al. 2005), validity of Allen's rule in leg lengths (Alho et al. 2011), as well as in studies of variation in energy storage patterns (Jönsson et al. 2009) and testis weight (Hettyey et al. 2005). Furthermore, studies in sex chromosome differentiation (Rodrigues et al. 2014), genome size variation (Matsuba and Merilä 2006), patterns of growth and age structure (Hjernquist et al. 2012), plasticity in age and size at metamorphosis (Merilä et al. 2000), embryonic and larval development and growth (Laugen et al. 2003; Merilä et al. 2004) have been conducted. They have also been subject to studies in genetic variation and differentiation (Palo et al. 2003, 2004), presence of persistent organic pollutants across Fennoscandia (ter Schure et al. 2002), larval nitrate tolerance (Johansson et al. 2001), and effect of UV-B radiation on embryos (Pahkala et al. 2002).

Many studies have also focused on age structure, longevity and body size variation among common frog populations (reviewed in: Miaud et al. 1999; Sinsch et al. 2015), but only few have focused on the small scale (i.e. at population and metapopulation level) variations in age and size structure (but see: Elmberg 1990; Augert and Joly 1993; Ryser 1996). Two recent studies (Alho et al. 2008; Patrelle et al. 2012a) have described the age structure of a common frog population living under extreme environmental conditions at the limit of species distribution range in the sub-Arctic Finland at 480 m a.s.l. Common frogs in this area occur also at altitudes higher than this, and a recent study discovered that microclimatic factors are

important determinants of species occurrence in this area (Blank et al. 2014). However, little is known about the life history of the common frogs inhabiting the high altitude sites in this area.

The aim of the present study was to compare adult life history traits related to age and size of common frogs from low ("valley" at 480 m a.s.l.) and nearby high ("hill" at 530-650 m a.s.l.) altitude sites in the sub-Artic Kilpisjärvi area.

Materials and methods

Study area

The study area was located at Kilpisjärvi ($69^0$03'N, $20^0$50'E), in the northwestern corner of Finland, 270 km north of the Arctic Circle and ca 50 km south-east of the Arctic Ocean. The climate in the area lies between the North Atlantic oceanic climate and the Eurasian continental climate, with a mean annual temperature of -2.0°C. The area experiences a large variation in solar radiation: in winter the sun is below the horizon for 55 days, while in summer there is no sunset for 62 days (Kauhanen 2013). The duration of the growth season varies between 69-132 days (mean 101 days; http://www.helsinki.fi/kilpis/english/Climate/records.htm; Järvinen 1987). The area resides about 100 km north of the continuous coniferous forest and belongs to the sub-alpine birch forest zone (*Betula pubescens*) at low altitudes (480-600 m). Above 600 m, the area is alpine tundra (Järvinen and Partanen 2008; Kauhanen 2013). Frogs in the area appear to be free of chytrid fungus (Patrelle et al. 2012b).

Data collection in the field

To obtain data for the high elevation sample (henceforth: hill-site), fieldwork was done during the summers of 2003 (9-28 August), 2009 (6-12 July) and 2010 (10-18 August) in the Malla nature reserve and the south-western slope of the Saana Mountain (Fig. 1). Adult animals were collected along transects

and data on 134 (81 females and 53 males) individuals were obtained. The captured animals were measured on site: body size was measured as the length from snout to vent (snout-vent length, SVL) to the nearest 0.1 mm using a dial caliper. Body mass was recorded with an electronic balance (Triton T2 400), with a precision of 0.01g. The animals were photographed and the second toe of the left arm was cut below the second phalange and stored in alcohol. The animals were then released at the site of capture. The exact location and altitude of the animals was taken with a hand-held Garmin GPS-tracker. Toes from at least 20 juveniles were also collected each year as they were required for calibration in skeletochronology (see below).

To obtain data for the low elevation sample (henceforth: valley-site), we re-used data from Patrelle et al. (2012a) which consisted of 169 adult frogs (116 females and 53 males) collected in the valley between the Malla and the Saana mountains (the distance separating the valley site and the hill sites is about 5 km) (Fig. 1). The raw data consisted of 699 individual measures (captures and recaptures) in different years. Excluding multiple recaptures from the same year, there were 397 unique captures during the five study years (1999-2003). Thus, recaptured individuals were considered repeatedly, in all years of capture or recapture, with SVL and body mass measured each time (if multiple recaptures in a single year, the latest date was considered), and age corrected based on the year of measurement. Animals were captured in late summer in the hill-site (i.e. post reproduction season) whereas in valley-site the captures took place in the early spring (i.e. pre-breeding and breeding season).

Skeletochronology

Age related parameters were estimated through skeletochronology *sensu* Castanet and Smirina (1990), using some modifications following Rozenblut and Ogielska (2005). Skeletochronology allows for the reliable estimation of age of individuals in most populations and species (Sinsch 2015), limited only in the correct assessment of age in old individuals (Wagner et al. 2011). In brief, the second phalange was decalcified for 3 h with 5% HNO_3, followed by inclusion in TissueTek, freezing and sectioning with a

Tehsys CR 3000 cryotome at 16 µm. The cross sections were stained with Ehrlich's haematoxylin for 3 hrs and photographed thereafter using an Olympus CX 31 microscope and Quick Photo Micro 2.3 software. We estimated age from the Lines of Arrested Growth (LAGs), after correction for resorption in older individuals by using cross sections from juveniles and subadults as recommended by Hemelaar (1985), Castanet and Smirina (1990), and Rozenblut and Ogielska (2005). Three independent observers (RP, DR and DC) counted the lines of arrested growth (LAGs) in 2–6 sections per individual.

The distance between two LAGs is an indicator of individual growth in a given age, and a pattern of decreasing intervals between LAGs after a few years is thought to indicate the onset of sexual maturity, with resources being reallocated from growth to reproduction (Smirina 1994). We inferred the age of sexual maturity from the bone growth pattern as indicated by Smirina (1994), and in addition compared with the youngest adult age class in our sample, considered as the minimal age of sexual maturity. Age was assessed in a similar way in the valley site (Patrelle et al. 2012a), except for endosteal resorption that was based on a method developed by Alho (2004). Since frogs in the valley were collected from breeding sites, all individuals were mature and age of sexual maturity was estimated as the minimum age in the sample (Patrelle et al. 2012a). Although slightly different approaches were used to account for endosteal resorption in hill and valley datasets, this is unlikely to have influenced age assessment.

Data analyses

We analyzed data from both the valley sites collected during five consecutive years (1999-2003; Patrelle et al. 2012a), and from the hill sites at three different time points (2003, 2009 and 2010). We computed a sexual dimorphism index (SDI) for SVL, body mass and average age, with the results arbitrarily defined as positive when females are the larger sex and negative in the converse situation (Lovich and Gibbons 1992) despite criticisms on using this method (see Ranta et al. 1994) since it allows to compare all three parameters:

182 $$SDI = \frac{Mean\ size_{larger\ sex}}{Mean\ size_{smaller\ sex}}$$

183

184 The average (minimum) age of maturation (A_{mat}) is the average number of growing seasons experienced
185 before size at sexual maturity is reached. Longevity (A_{max}) is the highest recorded age.

186

187 We fitted growth curves based on the von Bertalanffy growth function

188

189 $$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

190

191 with the FSA package in R (Ogle 2010), where L_t is the body size at age t (or after t growing seasons
192 experienced), L_∞ is the estimated asymptotic body size, k is a growth coefficient relating the rate of decline
193 in growth as individuals attain maximum body size (units are year^{-1}), and t_0 is the age at metamorphosis.
194 Differences between sites and sex-specific growth rates were tested using a likelihood ratio test (Kimura
195 1980) using package fishmethods in R (Nelson 2014).

196

197 Adult survival rate was calculated according to Robson and Chapman's (1961, in Krebs 1989) formula: S
198 $= T/\sum N+T-1$ with the fishmethods package. Here, S is the average finite survival rate, T is the sum of the
199 coded ages times their frequencies when age is found by setting the youngest included age-class to 0, the
200 next age to 1 and so forth $T= 0N_x+1N_{x+1}+2N_{x+2}+...iN_{x+i}$; $\sum N$ is the number of animals from age-class x to
201 $x+1=N_x+N_{x+1}+N_{x+2}+...+N_{x+i}$; and N_x is the number of individuals in age-class x. This formula is only
202 valid for stationary populations in which recruitment and survival are relatively constant. As these
203 assumptions are rarely met in nature, calculated values should be seen as relative estimates allowing only
204 for comparisons between sexes and sites (Schabetsberger and Goldschmid 1994). We note that for the
205 valley site, the assumption about similar survival rates between sexes has been verified (Alho et al. 2008).

Growth rate (R) can be calculated with the following equation: $R = dL/dt = k \times (L_\infty - L_t)$, which is maximal when L_t is minimal.

SVL and body mass were modeled as a function of site, sex, year and age using general additive Mixed Models (GAMMs) which enable individual effects to be accommodated in analyses, thus accounting for non-independence among individuals at the same site or year. The 'mgcv' package (Wood 2006) was used to fit the models to the data in R. Pairwise comparisons between site, sexes and years were made using the R multcomp package (Hothorn et al. 2008).

Results

All the age, size and growth related parameters are summarized in Table 1. There were significant differences in body size between sites, sexes and years (Table 2). Frogs were significantly larger and heavier in the valley as compared to the hill site (Table 3, Appendix 1). Females were significantly heavier, but not significantly larger in SVL than males (Table 4). Significant differences in body size were observed also among years (Table 2, Appendix 1).

Females from the hill site were significantly smaller and lighter compared to females from the valley site (Table 3, Fig. 2A, B): on average females from the hill-site had 78% and 47% of the SVL and body mass of the valley females, respectively. Likewise, males from the hill site were smaller and lighter compared with males from the valley site (Table 3, Fig. 2A, B, Appendix 1): on average, the hill-site males had 90% and 66% of the SVL and body mass of valley males, respectively. In both sexes, SVL and body mass were significantly correlated within each of the sites (Table 4). The regression equations for females had very similar slopes but different intercept values, indicating the females from the hill site had a smaller body mass compared with females from the valley site for the same body length value (Table 4).

232

233 The degree of SDI for SVL and body mass varied between the sexes in both sites: in the valley site
234 females were larger (SDI_{SVL} = 1.08) and heavier ($SDI_{body\ mass}$ = 1.23), while in the hill site males were
235 larger (SDI_{SVL} = -1.05) and heavier ($SDI_{body\ mass}$ = -1.14). The sex ratio was biased in both sites, with
236 females being more abundant. The sex ratio (males: females) in the hill site was 0.65 (53:81), significantly
237 higher as compared to the valley site 0.45 (53:116) (*chi-square* = 3.942, *d.f.* = 1, p = 0.047). The SDI for
238 mean age showed that females were older in both sites, but the differences between sexes were much
239 larger in the valley population (valley: $SDI_{mean\ age}$ = 1.39; hill: $SDI_{mean\ age}$ = 1.08).
240

241 Both females and males from the valley site were significantly older than individuals from the hill site
242 (Table 3, Fig. 2C, Appendix 1). Individuals from the hill site had an average age of only 50% in the case
243 of females and 64% for males, compared to those in the valley site.
244

245 The growth curves were significantly different between the hill and valley sites (Table 5, Fig. 3) although
246 there were no significant differences between any of the Von Bertalanffy parameters. Females and males
247 showed significantly different growth curves (Table 5, Fig. 4) driven by significant differences in t_0 (p
248 <0.001) and growth coefficient (p = 0.039). Differences in L_∞ were substantial, but not significant (p =
249 0.89). In both hill and valley sites frogs showed an evident increase in body length and body mass (Table
250 2, Fig. 2A - C) but frogs reached an asymptote in length and body mass only in hill site (Fig. 4A, B).
251

252 Age structure differed between sites and sexes (Fig. 5, Appendix 1). For the hill site the most abundant
253 age class for both females and males was 6 years old (48% of females and 34% of males). There are
254 differences among years in the age structure, with a decrease in age composition in 2009 compared to
255 2003, followed by an increase from 2009 to 2010 (Fig. 6). For valley site, the proportion of females 10
256 years old (23%) was the highest, whereas the highest proportion (28%) of males were five year old (Fig.
257 5).

The lower average age of the frogs on the hill site is supported by the fact that none of the 258 individuals toe-clipped in 2003 were recaptured in 2009. In 2010 19% of the individuals marked in 2009 were recaptured (14 individuals out of 143 captures, not including the 25 freshly metamorphosed individuals).

Discussion

Our study revealed significant differences in common frog life-history traits, including life span, survival, body size and mass over short spatial and temporal scales. Individuals from the hill site had shorter life span, both in terms of mean age and longevity, smaller body size and body mass than those from the valley-site. These differences were more pronounced in females as compared to males. Males were larger and heavier than females in the hill than in the valley site indicating that females were more affected by factor(s) associated with higher altitude conditions than males. The individuals from the hill site exhibited strong interannual variation in body mass and length, but mostly in the former, and also in age structure. In the following, we discuss these findings in relation to what is known about common frog life history trait differentiation, in particular, in relation to environmental gradients.

Studies of neighboring populations at small spatial scale often are focused on local ecological conditions as the main explanation for the observed differences (Miaud and Merilä 2001). A recent review (Sinsch et al. 2015) found that common frogs at higher altitudes and latitudes (i.e. experiencing adverse conditions) reached reproductive maturity at significantly older ages and enjoyed an increased longevity as well. Our results support this finding only for the valley site frogs. This finding is at odds with Miaud et al. (1999), who showed that under adverse conditions *R. temporaria* grows older and larger, with sexual size dimorphism increasing with climatic harshness, due to slower juvenile growth rate and a delayed maturity in females. One possible explanation for this difference is that conditions at the hill-site are so adverse that common frogs there never reach very old ages and sizes.

283 The hill sites are likely to be harsher environments than the valley sites as ambient temperatures drop with
284 altitude on average 0.9^0C with every 100 m increase in altitude (Järvinen 1989), exposure to winds
285 becomes higher, and vegetation cover is reduced (Kauhanen 2013). Because of the latter, also exposure to
286 predation by birds and mammals is likely to increase. Likewise, lower invertebrate prey abundance should
287 make finding food more difficult in the hill as compared valley sites. All these coupled with the slightly
288 shorter growth season in the hill than in valley sites should impose constraints on growth, development
289 and survival (Muir et al. 2014). In contrast, although day length is the most accurate and consistent
290 environmental cue in northernmost seasonal environments (Saikkonen et al. 2012), and has a major
291 influence on mean age, age at first reproduction and longevity in common frog (Hjernquist et al. 2012), it
292 cannot explain the life history trait differentiation between hill and valley sites situated just a few
293 kilometers apart.
294
295 Temperature has a strong effect on the development and growth of most living organisms (Marchand
296 2014). A larger body size and lower growth rates in adults are adaptive in colder environments (Angilletta
297 et al. 2004), and are probably coupled with higher metabolic rates required to sustain activity during the
298 shorter growing season and lower predation risk. To reach the large body size in a cold environment,
299 individuals must prolong growth period and delay reproduction relative to those in warm environments.
300 Such delayed maturation is adaptive when larger body size favors an increase in fecundity and/or survival
301 rates (Stearns 1992; Angilletta et al. 2004). Contrary to Bergmann´s rule, body size variation in adult
302 common frogs is not linearly related to latitude (or altitude), but covaries with age as expected for a
303 species that grows indeterminately (Laugen et al. 2005; Sinsch et al. 2015). This indicates that variation in
304 common frog body size mirrors differences in age structure. Thus, the finding that individuals from the
305 hill sites had lower age and smaller body size than those from the valley-sites fits with this idea. However,
306 an alternative explanation for age (and size) differences between hill and valley relates to how the data
307 from the contrasting sites were collected. Since the hill sites were sampled in the late summer and valley
308 sites in early summer, the hill samples may include more subadults than the valley samples which were all

breeding adults. However, we believe this in an unlikely explanation for the observed differences because the differences in body size persisted even after controlling for age difference among sites.

We discovered that the population sex ratio differed significantly between the hill and valley site, with stronger bias towards females in the valley than in the hill site. The strong female bias in the valley population is known since earlier (Alho et al. 2008, 2010), with on average, only one third of breeding individuals being males. The cause of this sex-bias is thought be environmentally induced sex reversal for which evidence has been accumulating (Perrin 2009; Matsuba et al. 2010; Rodrigues et al. 2016). The reason why the sex bias is less pronounced in the hill site than in the valley site is not obvious, but this difference can be related to differences in environmental conditions experienced by maturing individuals. Likewise, an earlier study has confirmed that males and females in the valley site do not differ in their survival rates (Alho et al. 2008). Hence, the differences in sex ratio among valley and hill sites are unlikely to depend on sex differences in mortality.

In conclusion, the results demonstrate marked differences in common frog key life history traits between ecologically contrasting environments in the sub-Arctic. The frogs inhabiting the environmentally more adverse hill sites are younger and smaller than those inhabiting valleys. As these findings go against the large-scale patterns showing that common frogs tend to get older with increasing environmental adversity (Miaud et al. 1999; Sinsch et al. 2015), the results highlight the importance of scale in ecology (Schneider 2001): downscaling large scale patterns may be of limited validity.

Acknowledgements

Fieldwork was made possible thanks to three grants from Lapland Biosphere-Atmosphere Facility Finland Programs Lapbiat (2003) and Lapbiat 2 (2009 and 2010), funded by the EU. Collecting permits were

provided by Lapland Regional Environmental Center (permit no. LAP-2009-L-356-254) and Lapland Center for Economic Development, Transport and the Environment (permit no. LAPELY/926/07.01.2010). The skeletochronological procedure was approved by the Ethics Committee of the Faculty of Natural and Agricultural Sciences, Ovidius University, Constanţa on 19.06.2013. Special thanks to Ruşti Dorel, Dr. Ioan Ghira, Jianu Claudia and Dr. Tudor Marian for their help with fieldwork, to Dr. Tibor Kovacs for the valuable advice and support during the fieldwork, and to Dr. Antero Järvinen that provided constant support and advice.

References

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. Evol Appl 1:95–111. doi:10.1111/j.1752-4571.2007.00013.x

Alho JS (2004) Population biology of the common frog in subarctic. MSc Thesis, University of Helsinki

Alho JS, Herczeg G, Merilä J (2008) Female-biased sex ratios in subarctic common frogs. J Zool 275:57–63. doi: 10.1111/j.1469-7998.2007.00409.x

Alho JS, Matsuba C, Merilä J (2010) Sex reversal and primary sex ratios in the common frog (*Rana temporaria*). Mol Ecol 19:1763–1773. doi: 10.1111/j.1365-294X.2010.04607.x

Alho JS, Herczeg G, Laugen AT, Räsänen K, Laurila A, Merilä J (2011) Allen's rule revisited: quantitative genetics of extremity length in the common frog along a latitudinal gradient. J Evol Biol 24:59–70. doi: 10.1111/j.1420-9101.2010.02141.x

Angilletta Jr. MJ, Steury TD, Sears MW (2004) Temperature, growth rate and body size in ectotherms: fitting pieces of a life history puzzle. Integr Comp Biol 44:498–509. doi: 10.1093/icb/44.6.498

Augert D, Joly P (1993) Plasticity of age at maturity between two neighbouring populations of the common frog (*Rana temporaria* L.). Can J Zool 71:26–33. doi: 10.1139/z93-005

Blank L, Luoto M, Merilä J (2014) Potential effects of climate change on the distribution of the common frog *Rana temporaria* at its northern range margin. Isr J Ecol Evol 59:130–140. doi:10.1080/15659801.2014.888825

Bulgarella M, Trewick SA, Godfrey AJR, Sinclair BJ, Morgan-Richards M (2015) Elevational variation in adult body size and growth rate but not in metabolic rate in the tree weta *Hemideina crassidens*. J Insect Physiol 75:30–38. doi: 10.1016/j.jinsphys.2015.02.012

Castanet J, Smirina E (1990) Introduction to the skeletochronological method in amphibians and reptiles. Ann Sci Nat Zool (Paris) 11:191–196

Cogălniceanu D, Băncilă R, Plăiaşu R, Samoilă C, Hartel T (2012) Aquatic habitat used by amphibians with specific reference to *Rana temporaria* at high elevations (Retezat Mountains National Park, Romania). Ann Limnol - Int J Lim 48:355–362. doi:10.1051/limn/2012026

Elmberg J (1990) Long-term survival, length of breeding season, and operational sex ratio in a boreal population of common frogs, *Rana temporaria* L. Can J Zool 68:121–127. doi: 10.1139/z90-017

Endler JA (1977) Geographic variation, speciation, and clines (No. 10). Princeton University Press, Princeton

Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. Mol Ecol 17:167–178. doi:10.1111/j.1365-294X.2007.03413.x

Hemelaar ASM (1985) An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* and its application to populations from different latitudes and altitudes. Amphib-Reptilia 6:343–353. doi: 10.1163/156853885X00326

Hettyey A, Laurila A, Herczeg G, Jönsson KI, Kovács T, Merilä J (2005) Does testis weight decline towards the Subarctic? A case study on the common frog, *Rana temporaria*. Naturwissenschaften 92:188–192. doi: 10.1007/s00114-005-0607-3

Hill JK, Griffiths HM, Thomas CD (2011) Climate change and evolutionary adaptations at species' range margins. Annu Rev Entomol 56:143–159. doi: 10.1146/annurev-ento-120709-144746

Hjernquist MB, Söderman F, Jönsson KI, Herczeg G, Laurila A, Merilä J (2012) Seasonality determines patterns of growth and age structure over a geographic gradient in an ectothermic vertebrate. Oecologia 170:641–649. doi: 10.1007/s00442-012-2338-4

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363. doi: 10.1002/bimj.200810425

Järvinen A (1987) Basic climatological data on the Kilpisjärvi area, NW Finnish Lapland. Kilpisjärvi Notes 10:1–16

Järvinen A (1989) The life history of *Ranunculus glacialis*, an Arctic-Alpine perennial herb, in Finnish Lapland. Holarctic Ecol 12:152–62. doi: 10.1111/j.1600-0587.1989.tb00834.x

Järvinen A, Partanen R (2008) Stand dynamics of mountain birch, *Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti in NW Finnish Lapland. Kilpisjärvi Notes 21:6–13

Johansson M, Räsänen K, Merilä J (2001) Comparison of nitrate tolerance between different populations of the common frog, *Rana temporaria*. Aquat Toxicol 54:1–14. doi:10.1016/S0166-445X(00)00182-x

Jönsson KI, Herczeg G, O'Hara RB, Söderman F, Ter Schure AF, Larsson P, Merilä J (2009) Sexual patterns of prebreeding energy reserves in the common frog *Rana temporaria* along a latitudinal gradient. Ecography 32:831–839. doi: 10.1111/j.1600-0587.2009.05352.x

Kauhanen HO (2013) Mountains of Kilpisjärvi host an abundance of threatened plants in Finnish Lapland. Bot Pac 2:43–52. doi: 10.17581/bp.2013.02105

Kimura DK (1980) Likelihood methods for the von Bertalanffy growth curve. US Fish Bull 77:765–776

Krebs CJ (1989) Ecological methodology. Harper and Row, New York

Kuzmin S, Ishchenko V, Tuniyev B, Beebee T, Andreone F, Nyström P, Anthony B, Schmidt B, Ogrodowczyk A, Ogielska M, Bosch J, Miaud C, Loman J, Cogălniceanu D, Kovács T, Kiss I (2009) *Rana temporaria*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Accessed 11 March 2015

438 Laugen AT, Laurila A, Jönsson I, Söderman F, Merilä J (2005) Do common frogs (*Rana temporaria*)
439 follow Bergmann's rule? Evol Ecol Res 7:717–731
440
441 Laugen AT, Laurila A, Merilä J (2003) Latitudinal and temperature-dependent variation in embryonic
442 development and growth in *Rana temporaria.* Oecologia 135:548–554. doi: 10.1007/s00442-003-1229-0
443
444 Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annu
445 Rev Ecol Syst 27:237–277. doi: 10.1146/annurev.ecolsys.27.1.237
446
447 Lovich JE, Gibbons JW (1992) A review of techniques for quantifying sexual size dimorphism. Growth
448 Dev Aging 56:269–281
449
450 Ludwig G, Sinsch U, Pelster B (2015) Behavioural adaptations of *Rana temporaria* to cold climates. J
451 Therm Biol 49:82–90. doi: 10.1016/j.jtherbio.2015.02.006
452
453 Marchand PJ (2014) Life in the cold: an introduction to winter ecology. University Press of New England,
454 Lebanon, New Hampshire
455
456 Matsuba C, Merilä J (2006) Genome size variation in the common frog *Rana temporaria*. Hereditas
457 143:155–158. doi: 10.1111/j.2006.0018-0661.01919.x
458
459 Matsuba C, Alho JS, Merilä J (2010) Recombination rate between sex chromosomes depends on
460 phenotypic sex in the common frog. Evolution 64:3634–3637. doi: 10.1111/j.1558-5646.2010.01076.x
461
462 Mayr E (1963) Animal species and their evolution. Belknap Press, Harvard
463

Merilä J, Laurila A, Laugen AT, Räsänen K, Pahkala M (2000) Plasticity in age and size at metamorphosis in *Rana temporaria* - comparison of high and low latitude populations. Ecography 23:457–465. doi: 10.1111/j.1600-0587.2000.tb00302.x

Merilä J, Laurila A, Lindgren B (2004) Variation in the degree and costs of adaptive phenotypic plasticity among *Rana temporaria* populations. J Evol Biol 17:1132–1140. doi: 10.1111/j.1420-9101.2004.00744.x

Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol Appl 7:1–14. doi: 10.1111/eva.12137

Miaud C, Guyétant R, Elmberg J (1999) Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. J Zool 249:61–73. doi: 10.1111/j.1469-7998.1999.tb01060.x

Miaud C, Merilä J (2001) Local adaptation or environmental induction? Causes of population differentiation in alpine amphibians. Biota 2:31–50

Moritz C, Agudo R (2013) The future of species under climate change: resilience or decline? Science 341:504–508. doi: 10.1126/science.1237190

Morrison C, Hero JM (2003) Geographic variation in life-history characteristics of amphibians: a review. J Anim Ecol 72:270–279. doi: 10.1046/j.1365-2656.2003.00696.x

Muir AP, Biek R, Mable BK (2014) Local adaptation with gene flow: temperature parameters drive adaptation to altitude in the common frog (*Rana temporaria*). Mol Ecol 23:561–574. doi: 10.1111/mec.12624

Nelson GA (2014) Package 'fishmethods' in R. http://cran.rproject.org/web/packages/fishmethods/fishmethods.pdf. Accessed 20 March 2015

Ogle DH (2010) FSA - package in R. http://www.rforge.net/FSA/index.html. Accessed 20 March 2015

Pahkala M, Laurila A, Merilä J (2002) Effects of ultraviolet-B radiation on common frog *Rana temporaria* embryos from along a latitudinal gradient. Oecologia 133:458–465. doi: 10.1007/s00442-002-1058-6

Palo JU, O'Hara RB, Laugen AT, Laurila A, Primmer CR, Merilä J (2003) Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. Mol Ecol 12:1963–1978. doi: 10.1046/j.1365-294X.2003.01865.x

Palo JU, Schmeller DS, Laurila A, Primmer CR, Kuzmin SL, Merilä J (2004) High degree of population subdivision in a widespread amphibian. Mol Ecol 13:2631–2644. doi: 10.1111/j.1365-294X.2004.02269.x

Patrelle C, Hjernquist MB, Laurila A, Söderman F, Merilä J (2012a) Sex differences in age structure, growth rate and body size of common frogs *Rana temporaria* in the subarctic. Polar Biol 35:1505–1513. doi:10.1007/s00300-012-1190-7

Patrelle C, Miaud C, Cristina N, Kulberg P, Merilä J (2012b) Chytrid fungus screening in a population of common frogs from Northern Finland. Herpetol Rev 43:422–425

Perrin N (2009) Sex reversal: a fountain of youth for sex chromosomes? Evolution 63:3043–3049. doi: 10.1111/j.1558-5646.2009.00837.x

Plăiaşu R, Băncila RI, Cogălniceanu D (2010) Body size variation in *Rana temporaria* populations inhabiting extreme environments. Ovidius Univ Ann Nat Sci Biol Ecol Ser 14:121–126

Ranta E, Laurila A, Elmberg J (1994) Reinventing the wheel: analysis of sexual dimorphism in body size. Oikos 70:313–321. doi: 10.2307/3545768

Rodrigues N, Merilä J, Patrelle C, Perrin N (2014) Geographic variation in sex-chromosome differentiation in the common frog (*Rana temporaria*). Mol Ecol 23:3409–3418. doi: 10.1111/mec.12829

Rodrigues N, Vuille Y, Brelsford A, Merilä J, Perrin N (2016) The genetic contribution to sex determination and number of sex chromosomes vary among populations of common frogs (*Rana temporaria*). Heredity 117:25–32. doi:10.1038/hdy.2016.22

Roff DA (1992) Evolution of life histories: theory and analysis. Chapman and Hall, New York

Rozenblut B, Ogielska M (2005) Development and growth of long bones in European water frogs (Amphibia: Anura: Ranidae), with remarks on age determination. J Morphol 265:304–317. doi: 10.1002/jmor.10344

Ryser J (1996) Comparative life histories of a low-and a high-elevation population of the common frog *Rana temporaria*. Amphibia-Reptilia 17:183–195. doi: 10.1163/156853896X00379

Saikkonen K, Taulavuori K, Hyvönen T, Gundel PE, Hamilton CE, Vänninen I, Nissinen A, Helander M (2012) Climate change-driven species' range shifts filtered by photoperiodism. Nat Clim Chang 2:239–242. doi: 10.1038/nclimate1430

Schabetsberger R, Goldschmid A (1994) Age structure and survival rate in the Alpine newts (*Triturus alpestris*) at high altitude. Alytes 12:41–47

Schneider DC (2001) The rise of the concept of scale in ecology. BioScience 51:545–553. doi: 10.1641/0006-3568(2001)051[0545:trotco]2.0.co;2

Sinsch U (2015) Review: Skeletochronological assessment of demographic life-history traits in amphibians. Herp J 25:5–13.

Sinsch U, Pelster B, Ludwig G (2015) Large-scale variation of size and age-related life-history traits in the common frog: a sensitive test case for macroecological rules. J Zool 297:32–43. doi: 10.1111/jzo.12243

Smirina EM (1994) Age-determination and longevity in amphibians. Gerontology 40:133–146. doi: 10.1159/000213583

Stearns SC (1992) The Evolution of Life Histories. Oxford University Press, Oxford

Ter Schure AF, Larsson P, Merilä J, Jönsson KI (2002) Latitudinal fractionation of polybrominated diphenyl ethers and polychlorinated biphenyls in frogs (*Rana temporaria*). Environ Sci Technol 36:5057–5061. doi: 10.1021/es0258632

Van Buskirk J, Arioli M (2005) Habitat specialization and adaptive phenotypic divergence of anuran populations. J Evol Biol 18:596–608. doi: 10.1111/j.1420-9101.2004.00869.x

Wagner A, Schabetsberger R, Sztatecsny M, Kaiser R (2011) Skeletochronology of phalanges underestimates the true age of long-lived Alpine newts (*Ichthyosaura alpestris*). Herp J 21:145–148

Wood SN (2006) Generalized additive models: an introduction with R. Chapman and Hall/CRC Press, Boca Raton

571 Figure Captions

572

573 **Fig. 1** Location of the study site in northern Finland (inserted map) and of the valley and hill sites in
574 Kilpisjärvi (larger map).

575

576 **Fig. 2** Mean (A) SVL, (B) body mass and (C) age of female (white) and male (black) in common frogs,
577 *Rana temporaria*, from hill and valley sites

578

579 **Fig. 3** Body size a function of age as estimated by GAMMSs (generalized additive mixed models) for the
580 common frogs, *Rana temporaria* from: hill site, (A) SVL (mm) and (B) body mass (g) and valley site, (C)
581 SVL (mm) and (D) body mass (g); gray shading shows 95% confidence intervals

582

583

584 **Fig. 4** Length-at-age growth curves for the common frogs, *Rana temporaria*, derived with Von
585 Bertalanffy method. (A) Hill (open circle, solid line) and valley (closed circle, dotted line) sites, and (B)
586 females (open circle, solid line) and males (closed circle, dotted line)

587

588 **Fig. 5** Age distribution of female and male common frogs, *Rana temporaria*, from hill (n = 134) and
589 valley (n = 169) sites

590

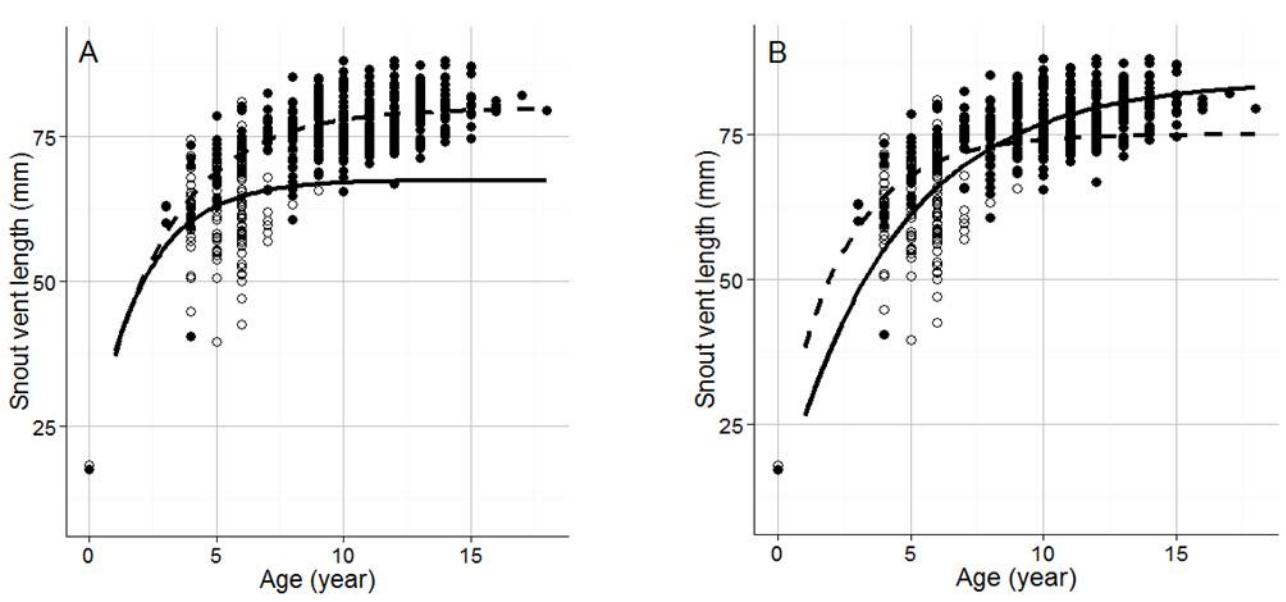
591 **Fig. 6** Yearly variation in age distribution of female and male common frogs *Rana temporaria* from the
592 hill site

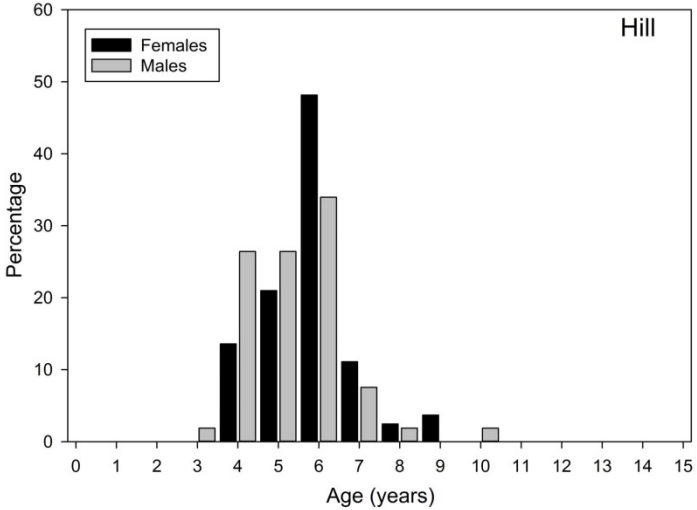
Table 3 The pairwise comparisons between site and sex on the SVL, body mass and age for the common frogs, *Rana temporaria*

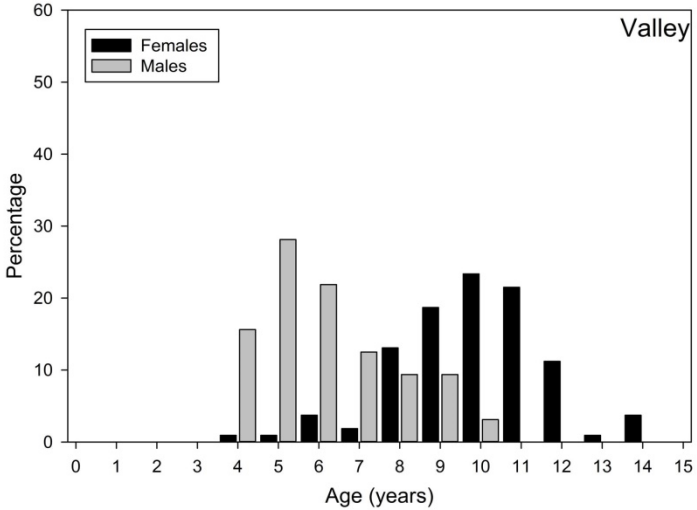
	Estimate	*SE*	*z*	*p*
SVL (mm)				
Valley Female - Hill Female	16.799	0.717	23.443	<0.001
Hill Male - Hill Female	3.675	1.001	3.671	0.001
Valley Male - Hill Female	10.702	0.819	13.067	<0.001
Hill Male - Valley Female	-13.124	0.850	-15.435	<0.001
Valley Male - Valley Female	-6.097	0.626	-9.744	<0.001
Valley Male - Hill Male	7.027	0.938	7.490	<0.001
Body mass (g)				
Valley Female - Hill Female	25.628	1.118	22.914	<0.001
Hill Male - Hill Female	3.216	1.566	2.054	0.162
Valley Male - Hill Female	16.431	1.272	12.916	<0.001
Hill Male- Valley Female	-22.411	1.328	-16.879	<0.001
Valley Male - Valley Female	-9.197	0.965	-9.535	<0.001
Valley Male - Hill Male	13.214	1.460	9.053	<0.001
Age (year)				
Valley Female - Hill Female	5.713	0.263	21.736	<0.001
Hill Male - Hill Female	-0.451	0.368	-1.224	0.603
Valley Male - Hill Female	2.516	0.299	8.411	<0.001
Hill Male- Valley Female	-6.164	0.312	-19.750	<0.001
Valley Male - Valley Female	-3.198	0.227	-14.108	<0.001
Valley Male - Hill Male	2.966	0.343	8.643	<0.001

Table 5. Likelihood ratio test results comparing the von Bertalanffy model parameters between (1) hill and (2) valley sites, as well as between (3) female and (4) male common frog, *Rana temporaria*.

Hypothesis	*Chi-sq.*	*df*	*p*
Site			
$L_{\infty 1}=L_{\infty 2}$	1.92	1	0.166
$k_1=k_2$	2.5	1	0.114
$t_{01}=t_{02}$	0	1	1
$L_{\infty 1}=L_{\infty 2}$, $k_1=k_2$, $t_{01}=t_{02}$	32.53	3	**<0.001**
Sex			
$L_{\infty 3}=L_{\infty 4}$	0.02	1	0.888
$k_3=k_4$	4.28	1	0.039
$t_{03}=t_{03}$	13.23	1	**<0.001**
$L\infty_3=L\infty_3$, $k_3=k_4$, $t_{03}=t_{03}$	33.84	3	**<0.001**


A
B
Snout vent length (mm)
Age (year)
25
50
75
0
5
10
15


Hill
Females
Males
Percentage
Age (years)
0
10
20
30
40
50
60
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15


Valley
Females
Males
Percentage
Age (years)
0
10
20
30
40
50
60
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15